PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Campbell et al.	)	Group Art Unit: 1632
	)	
Application No.: 09/225,233	)	Examiner: D. Crouch
	)	
Filed: January 4, 1999	)	Confirmation No.: 2711
	)	
For: QUIESCENT CELL POPULATIONS FOR NUCLEAR TRANSFER	)	
	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF IRINA A. POLEJAEVA, Ph.D, UNDER 37 C.F.R. § 1.132

I, Irina A. Polejaeva, declare that:

1. Since December of 2003, I have held the position of Vice President of Assisted Reproductive Technology & Chief Scientific Officer, ViaGen Inc. I held the position of Director of Assisted Reproductive Technology between October 2002 and November 2003. At ViaGen Inc, I have been involved in cloning horses, cattle, and pigs using somatic cells. A copy of my CV is attached.

2. I understand that ViaGen Inc. is a licensee of the above-referenced patent application from Start Licensing Inc.

3. I further understand that the prosecution of the above-referenced patent application is controlled by Start Licensing Inc., a joint venture between Geron Corporation and Exeter Life Sciences Inc.

4. I further understand that ViaGen Inc. is a subsidiary of Exeter Life Sciences Inc.

5. In 1998-2002, I was the Head of Cell Biology, Senior Scientist, and Porcine Nuclear Transfer Project Manager at PPL Therapeutics Inc. I was responsible for planning, budgeting, training, coordination, evaluating, and reporting of all aspects of research projects in the area of Cell Biology and Porcine Embryology. This included developing nuclear transfer procedures in order to produce transgenic mammals and improving efficiency of nuclear transfer procedures by optimizing oocyte activation. I was responsible for designing appropriate tissue culture conditions for generation and propagation of primary bovine and porcine cells for nuclear transfer programs.

6. In 1996-1998, I was a Scientist and Project Manager for porcine stem cell project at PPL Therapeutics Inc. I was responsible for planning, conducting, supervising, and summarizing data on a porcine stem cell project.

7. In 1993-1996, I was a postdoctoral fellow at Utah State University. During my research at Utah State University, I established bovine, mink, and ovine ES cell lines. I optimized culture conditions for the isolation and maintenance of bovine ES cells. I was further involved in a project that measured DNA methylase activity during various passages of bovine ES cells.

8. In 1993, I was a Scientist in the Department of Biotechnology, National Research Institute of Animal Science, Russia. I was involved in a project aimed at establishing bovine ES cells. I established primary cell cultures from lung, liver, kidney and connective tissue of transgenic rabbits for investigating bovine growth hormone gene expression.

9. In 1989-1992, I was a student at the Laboratory of Cell Engineering (lab of Dr. N. Strelchenko) at the National Institute of Animal Science, Russia. I was involved in several projects aimed at establishing mouse ES cells and the production of transgenic murine ES cells using a retroviral vector. I established primary cultures of mammalian and insect cells, prepared feeder layers for ES cell maintenance, maintained and transformed embryonic stem cells, and mastered procedures for freezing and thawing cells as well as karyotyping cell lines. In June 1993, I was granted a Ph.D. in Developmental Biology/Embryology, from the Department of Biotechnology, National Research Institute of Animal Science, Dubrovitsy, Moscow region, Russia.

10. In June 1985, I obtained a Master of Science in Animal Science at the Kubanski Agricultural Institute, Krasnodar, Russia.

11. I have published numerous manuscripts in the area of cloning mammals from somatic cells using nuclear transfer. Representative manuscripts are: Walker S.C., Christenson R.K., Ruiz R.P., Reeves D.E., Pratt S.L., Arenivas F., Williams N.E., Bruner B.L., Polejaeva I.A., Comparison of meat composition from offspring of cloned and conventionally produced boars, *Theriogenology* 67:178-184 (2007); Irina A. Polejaeva, Pig cloning: advances and applications, *Reproduction, Supplement* 58: 293-300 (2001); and Irina A. Polejaeva, Shu-Hung Chen, Todd D. Vaught, Raymond L. Page, June Mullins, Yifan Dai, Jeremy Boone, Shawn Walker, Dave Ayares, Alan Colman and Keith H. S. Campbell, Cloned Piglets Produced by Nuclear Transfer from Adult Somatic Cells, *Nature* 407: 86-90 (2000).

12. Through my experience, I have gained knowledge of many aspects of mammalian biology, including nuclear transfer (cloning) technology; oocyte activation

and early embryo development after nuclear transfer; mechanism of nuclear reprogramming; early events of mammalian development; gene transfer based on the use of somatic cell nuclear transfer and embryonic stem cell technologies; gene targeting in ES cells and somatic cells; *in vitro* differentiation of cells; and the developmental biology and embryology of mammals.

Campbell's U.S. Patent Application No. 09/225,233

13. I have read Campbell's U.S. Patent Application No. 09/225,233 ("the Campbell '233 application"), a copy of which is attached hereto.

14. The Campbell '233 application states: "It has now been found that quiescent cells, that is to say cells that are not actively proliferating by means of the cell cycle, can advantageously used as nuclear donors in the reconstitution of an animal embryo. Such animals may then be allowed to develop to term." (At 4, lines 27-31.)

15. Based on my reading of the Campbell '233 application, the basic technique of somatic cell nuclear transfer taught by the Campbell '233 application involves transferring the nuclear genetic material of a quiescent somatic cell (the nuclear donor cell) into a suitable recipient cell.

16. In the Campbell '233 application, a nuclear donor cell can be in the G_0 stage of the cell cycle. (At 9, lines 14-28.)

17. In the Campbell '233 application, a suitable recipient cell can be an enucleated oocyte in the metaphase II stage of the cell cycle. (At 11, lines 22-35.)

18. The Campbell '233 application teaches many permutations of this technique. For example, on pages 9-13, the Campbell '233 application teaches various

techniques for introducing the nuclear genetic material of a somatic cell into a recipient cell.

19. Similarly, on pages 14-15, the Campbell '233 application teaches various techniques for activating a reconstituted embryo.

20. Also, on pages 15-16, the Campbell '233 application teaches various techniques for increasing the number of cloned mammals, including the technique of serial nuclear transfer. Serial nuclear transfer involves performing an additional nuclear transfer using cells generated from the first nuclear transfer as donors for a second nuclear transfer.

21. The Campbell '233 application points out that most of the reconstituted embryos do not reprogram. (At 16, lines 5-9.)

22. This means that somatic cell nuclear transfer is an inefficient process. Many reconstituted embryos need to be created to generate a single viable embryo.

23. Based on the Campbell '233 application, I would have known in February of 1997 that somatic cell cloning of mammals, including pigs, by nuclear transfer was an inefficient process.

Polejaeva et al. (2000)

24. I have read an article by I.A. Polejaeva, S. Chen, T.D. Vaught, R.L. Page, J. Mullins, S. Ball. Y. Dal, J. Boone, S. Walker, D. Ayares, A. Colman, and K.H.S. Campbell entitled "Cloned pigs produced by transfer from adult somatic cells" in *Nature* 407:86-90 (2000)("Polejaeva et al. (2000)"), a copy of which is attached hereto.

25. I am one of the authors of Polejaeva et al. (2000).

26. Polejaeva et al. (2000) reports using a serial nuclear transfer technique to generate cloned pigs, in which the nucleus from the reconstructed embryo was transferred to an enucleated zygote for further development.

27. I was involved in the experiments that generated cloned pigs as reported in Polejaeva et al. (2000).

28. In my opinion, the serial nuclear transfer technique that we used to clone pigs in Polejaeva et al. (2000) is not required to generate cloned pigs. My opinion is based on my personal experience cloning pigs, and on many reports of successful cloning of pigs without this serial nuclear transfer technique.

29. In Polejaeva et al. (2000), we transferred 72 nuclear transfer embryos to a surrogate, which resulted in five live-born pigs. (At 87, Table 1.)

30. In Polejaeva et al. (2000), we indicated that greater than 4 good quality embryos are required to induce and maintain pregnancy in pigs, citing a paper published in 1966. (At 86, col. 2, paragraph 3.)

31. I have read the 1966 paper referenced in Polejaeva et al. (2000). It is an article by Polge et al. entitled "The effect of reducing the number of embryos during early stages of gestation on the maintenance of pregnancy in the pig" in *J. Reprod. Fert.* 12, 395-396 (1966)("Polge et al. (1966)"), a copy of which is attached hereto.

32. Polge et al. (1966) states: "These results suggest that more than four embryos are required in the pig in order to establish and maintain pregnancy." (At 397, first full paragraph.)

33. Thus, it was known prior to February of 1997 that more than four viable embryos in a single surrogate pig would be required after nuclear transfer to maintain pregnancy in the pig and result in live-born clones.

34. It would have been known from the Campbell '233 application that only a small fraction of nuclear transfer embryos were viable. (At 16, lines 5-9.)

35. Based on the Campbell '233 application and Polge et al. (1966), it would have been expected in February of 1997 that many nuclear transfer embryos would need to be transferred to a single pig to achieve success.

36. In Polejaeva et al. (2000), we indicated that, with the cell lines that did not result in live-born pigs, between 22 and 100 reconstructed embryos were transferred to each recipient female. (At 87, Table 1.)

37. In my opinion, the lack of live-born pigs with these cell lines may be due to not transferring enough reconstructed embryos to assure success. My opinion is based on my experience in cloning mammals, the required efficiency to produce live-born pigs, and the number of embryos transferred in Polejaeva et al. That is, we only transferred between 22 and 100 reconstructed embryos to each recipient female. I note that we transferred the reconstructed embryos approximately 1 day after nuclear transfer, at a time point when a smaller percentage of the embryos are likely to survive to term than those transferred at later time points. Since only a small percentage of reconstructed embryos actually develop into a live-born mammal and this efficiency can be less than one live-born mammal for every 300 reconstructed embryos (i.e., less than 0.33%;), the lack of any live births with these cell lines does not surprise me.

38. The '233 application provides a good example of the low efficiency of the production of viable embryos by nuclear transfer, teaching 1 live birth for 277 reconstructed sheep embryos. (At 34, table 4.)

39. Moreover, based on Table 1 of Polejaeva et al. (2000), it is evident that multiple hosts may be required in addition to transferring sufficient embryos. That is, one would not expect every recipient to get pregnant because of differences in the specific hosts.

40. We decided to pursue serial nuclear transfer to clone pigs because we thought that this approach would increase efficiency.

41. In fact, I have cloned pigs without serial nuclear transfer.

42. In my experience, transfer of 100-150 nuclear transfer pig embryos is normally sufficient to maintain pregnancies in pigs and produce cloned pigs by somatic cell nuclear transfer.

43. In my experience, transfer of less than 100-150 nuclear transfer pig embryos may be insufficient to maintain pregnancies in pigs and produce cloned pigs by somatic cell nuclear transfer.

44. In my experience, if the viability of the embryos is increased, for example, by the serial nuclear transfer technique described in Polejaeva et al. (2000), less than 100-150 nuclear transfer pig embryos may be sufficient to maintain pregnancies in pigs and produce cloned pigs by somatic cell nuclear transfer.

45. Many groups have published reports of cloning pigs without using serial nuclear transfer, including Onishi et al. (2000), Betthauser et al. (2000), and Lai et al. (2002).

Successful Cloning of Pigs without Serial Nuclear Transfer

46. I have read an article by A. Onishi, M. Iwamoto, T. Akita, S. Mikawa, K. Takeda, T. Awata, H. Hanada, and A.C.F. Perry entitled "Pig Cloning by Microinjection of Fetal Fibroblast Nuclei" in *Science* 289:188-189 (2000)("Onishi et al. (2000)"), a copy of which is attached hereto.

47. Onishi et al. (2000) successfully cloned pigs by nuclear transfer using somatic cells as nuclear donors. Onishi et al. (2000) did not use the serial nuclear transfer technique we used in Polejaeva et al (2000).

48. Onishi et al. (2000) states: "pigs typically require at least four fetuses for a successful pregnancy." (At 1188, col. 3, last full paragraph.)

49. Onishi et al. (2000) transferred 36 nuclear transfer embryos to the oviduct of a female surrogate to achieve a single offspring. (At 1189, col. 1-2, bridging paragraph.)

50. The cloning procedure used by Onishi et al. (2000) to successfully clone pigs using somatic cells follows the basic procedure in the Campbell '233 application.

51. The Campbell '233 application teaches that recipient cells for use in cloning can be enucleated oocytes in the metaphase II stage of the cell cycle. (At 12, lines 4-7.)

52. Onishi et al. (2000) used enucleated oocytes in the metaphase II stage of the cell cycle. (At 1190, col. 1, "18.")

53. The Campbell '233 application teaches that the donor nucleus can be in the G_0 stage at the time of nuclear transfer. (At 12, lines 15-17.)

54. Onishi et al. (2000) used confluent fibroblast cells in the G_0 stage of the cells cycle as nuclear donors. (At 1190, col. 1, "14.")

55. The Campbell '233 application teaches that the donor nuclei can by introduced into the enucleated oocyte by microinjection. (At 13, lines 31-35.)

56. Onishi et al. (2000) used microinjection to transfer fibroblast nuclei into enucleated oocytes. (At 1190, col. 2-3, "19.")

57. The Campbell '233 application teaches activating the reconstructed embryos subsequent to nuclear transfer. (At 11, lines 28-30.)

58. Onishi et al. (2000) maintained the oocytes for 4 hours after transfer before activating them. (*Id.*)

59. I have read an article by J. Betthauser, E. Forsberg, M. Augenstein, L. Childs, K. Eilertsen, J. Enos, T. Forsythe, P. Golueke, G. Jurgella, R. Koppang, T. Lesmeister, K. Mallon, G. Mell, P. Misica, M. Pace, M. Pfister-Genskow MN. Strelchenko, G. Voelker, S. Watt, S. Thompson, and M. Bishop entitled "Production of cloned pigs from in vitro systems" in *Nature Biotechnology* 18:1055-1059 (2000)("Betthauser et al. (2000)"), a copy of which is attached hereto.

60. Betthauser et al. (2000) successfully cloned pigs by nuclear transfer using somatic cells as nuclear donors. Betthauser et al. (2000) did not use the serial nuclear transfer technique we used in Polejaeva et al (2000).

61. Betthauser et al. (2000) transferred 115-164 nuclear transfer embryos to each recipient to achieve cloned pigs. (At 1057, col. 1, second full paragraph.)

62. Betthauser et al. (2000) concludes: "The large number of embryos (115-164; Table 1) that were used to produce the cloned piglets and additional pregnancies described here is consistent with low viability of NT embryos." (*Id.*)

63. I have read an article by L. Lai, D. Kolber-Simonds, K.W., Park, H.T. Cheong, J.L. Greenstein, G.S. Im, M. Samuel, A. Bonk, A. Rieke, B.N. Day, C.N. Murphy, D.B. Carter, R.J., Hawley and R.S. Prather entitled "Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning" in *Science* 295:1089-92 (2002)("Lai et al. (2002)"), a copy of which is attached hereto.

64. Lai et al. (2002) successfully cloned pigs by nuclear transfer using somatic cells as nuclear donors. Lai et al. (2002) did not use the serial nuclear transfer technique we used in Polejaeva et al (2000).

65. Lai et al. (2002) states: "A minimum of four viable embryos is required for establishment of pregnancy in pigs (27)." (At 1090, col. 3, first full paragraph.)

66. Lai et al. (2002) performed 20 transfers of only NT-derived embryos to unmated surrogates, which resulted in two pregnancies continuing to term. (At 1091, col. 1, first full paragraph.) In the two cases that went to term, 92 and 130 nuclear transfer embryos were transferred. (At 1090. Table 1.)

67. Based on Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experience cloning pigs, it is my opinion that serial nuclear transfer technique we used in Polejaeva et al. (2000) is not required for somatic cell cloning of pigs by nuclear transfer.

68. Based on Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs, it is my opinion that

somatic cell cloning of pigs by nuclear transfer in February of 1997 would not have required any technique that is not taught by the Campbell '233 application, or by the prior art.

69. Based on Polge et al. (1966), I would have further understood that somatic cell cloning of pigs by nuclear transfer would require having at least 4 viable pig embryos to maintain pregnancy.

70. Thus, in February of 1997, I would have expected that many nuclear transfer embryos would need to be transferred to a single pig to achieve successful somatic cell cloning of pigs.

71. Nonetheless, in February of 1997, I would have expected that transfer of sufficient nuclear transfer embryos to a single pig would result in successful somatic cell cloning of pigs.

72. The results of Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs confirm these expectations.

73. In my opinion, the somatic cell cloning of pigs exemplifies the broad applicability of the cloning techniques set forth in the Campbell '233 application. Many groups readily achieved the goal of cloning pigs.

74. Based on Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs, one can make many different modifications to the cloning techniques set forth in the Campbell '233 application, and still successfully clone pigs by somatic cell nuclear transfer.

75. Based on Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs, the important aspects of the successful cloning of pigs are set forth in the Campbell '233 application and the prior art.

76. Based on Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs, I would have expected that successful cloning of pigs could have been achieved with only routine experimentation in February of 1997 using the disclosure in the Campbell '233 application together with the knowledge in the art at that time.

77. It would have been routine in February of 1997 to design experiments based on the disclosure in the Campbell '233 application and the knowledge in the art at that time and to repeat the nuclear transfer experiments many times with hundreds of embryos and transfer those embryos to many different hosts. Nonetheless, many laboratories might not have the financial resources or manpower for such experiments.

Westhusin and Pennisi

78. I have read an article by M.E. Westhusin, C.R. Long, T. Shin, J.R. Hill, C.R. Looney, J.H. Pryor, and J.A. Piedrahita entitled "Cloning to reproduce desired genotypes" in *Theriogenology* 55:35-49 (2001)("Westhusin et al. (2001)"), a copy of which is attached.

79. Westhusin et al. (2001) makes a number of comments on the work effort required and the probability for producing a clone. (At 35-36.) Westhusin points out that the efficiency of each step of cloning varies among species, and that this affects the ease of which a particular animal can be cloned. (At 36.) Westhusin further points out

that the production of cloned pigs is very inefficient, and that the differences in the ability to clone a specific animal are simply a result of the time and resources that have thus far been invested in research for these species. (At 39.) Westhusin concludes that there is no solid evidence that suggests cloning will be limited to only a few specific animals, and in fact, most data collected to date suggests cloning will be applicable to a wide variety of different animals. (At 35.)

80. In my opinion, based on my experience in cloning mammals, there was no solid evidence in 2001 that any mammalian species could not be cloned, and there is no solid evidence today that any mammalian species cannot be cloned.

81. Based on the above passages, I understand that cloning is an inefficient process and a large number of oocytes may need to be reconstructed to achieve success. The probability for producing a clone increases proportionally with the number of oocytes reconstructed, but so does the "work effort," as well as the cost. Based on my experience in cloning mammals, the challenge for most laboratories in cloning mammals is one of having sufficient manpower and financial resources, since cloning of mammals is an expensive venture. The reconstruction of many oocytes for some species can involve large amounts of labor, albeit repetitive in nature, and high costs for infrastructure and personnel.

82. Based on my experience in cloning mammals, one way to maximize one's limited resources for cloning mammals is to improve the efficiency of the cloning process. Such improvements in cloning efficiency have been widely reported in the scientific literature, including many articles referenced herein. However, these improvements in efficiency are not strictly required for successful somatic cell nuclear

transfer; an alternative approach is to simply increase the overall number of reconstructed embryos transferred to recipients.

83. In my experience, successful clonings of previously-reported cloned species using increased numbers of reconstructed oocytes are not usually reported in publications, because they are not "publication worthy." These clonings are simply repeating what was already known.

84. Westhusin et al. (2001) states that "access to large numbers of oocytes at relatively low cost also provides the opportunity to carry out numerous attempts at cloning. Therefore, even if the overall efficiency is low, chances are given enough trial and enough embryos transferred, a clone of most any cow or bull could be produced." (At 37, first full paragraph.)

85. I understand that Westhusin is concluding that the successful cloning of a particular cow is virtually guaranteed by reconstructing a sufficient number of nuclear transfer embryos. That is, Westhusin is acknowledging that differences in efficiency can be compensated by reconstructing and transferring more nuclear transfer embryos.

86. Based on my experience cloning mammals, I agree with this statement, and believe that it applies to all species of mammals.

87. Westhusin et al. (2001) states: "Pigs represent an excellent example for pointing out that assisted reproduction technologies and techniques for nuclear transfer don't directly apply from one species to another." (At 39, second full paragraph.)

88. Westhusin does not explain what is meant by this passage. Westhusin may be referring to the fact that efficiency of cloning is relatively low in pigs. Westhusin

may also be referring to the fact that more than pigs have a particular requirement for four or more viable embryos to maintain pregnancy.

89. I have read an article by E. Pennisi and G. Vogel entitled "Clones: A hard Act to Follow" in Science 288:1722-1727 (2000)("Pennisi et al. (2000)"), a copy of which is attached.

90. Pennisi et al. (2000) indicates that several groups had difficulties cloning pigs, and indicates that the problem with cloning pigs was that "until 1998, few scientists had tried to work with immature pig eggs or to grow embryos in the lab. Pigs differ from cows and sheep in that they are born in litters, and unless there are at least four viable fetuses in the womb, the pregnancy fails. That means that a day's work has to yield at least several viable embryos if the cloning experiment is to have any chance of success." (At 1724-1725.)

91. In my opinion, Pennisi does not indicate that anything more than routine experimentation was required to clone pigs. Based on the Campbell '233 application, Pennisi et al. (2000), Polejaeva et al. (2000), Onishi et al. (2000), Betthauser et al. (2000), Lai et al. (2002), and my own experiences cloning pigs, it is my opinion that, once sufficient viable reconstructed nuclear transfer embryos were implanted in surrogate pigs, pig cloning was successful for many different groups. I would have understood in February of 1997, based on Polge et al. (1966), that somatic cell cloning of pigs by nuclear transfer would require having at least 4 viable NT embryos to maintain pregnancy, and that failure to reconstruct sufficient nuclear transfer embryos would likely lead to failure. In my opinion, the failure in the ability of some laboratories to clone pigs between 1997 and 2000 was likely due to these laboratories

reconstructing and/or implanting insufficient embryos to maintain pregnancy. In my opinion, these failures could have been remedied by reconstructing and transferring more nuclear transfer embryos to each surrogate pig.

DIFFERENCES BETWEEN A CLONE AND ITS DONOR MAMMAL

92. I have been asked to describe differences between a live-born clone of a pre-existing, non-embryonic, donor mammal and the donor mammal itself.

93. I understand that such a clone is currently claimed in the 'Campbell '233 application. I will refer to such a clone as "Applicant's clone."

94. Based on my experience cloning mammals, Applicant's clone is not made by nature.

95. Based on my experience cloning mammals, Applicant's clone can only be made by human intervention.

96. Based on my experience cloning mammals, Applicant's clone is not an exact copy of its donor mammal.

97. Based on my experience in mammalian reproduction, environmental factors would generate differences between Applicant's clone and its donor mammal.

98. Based on my experience cloning mammals, Applicant's clone could not exist before it was made.

99. Based on my experience cloning mammals, Applicant's clone occupies different space and time than its donor mammal, and is a time-delayed, inexact copy of its donor mammal. The clone contains the same genetic complement as its donor mammal, but is not an exact copy due to environmental differences during development.

100. The ability of Applicant's clone to exist at a time later than its donor mammal, but have the same genetic complement, is a markedly different characteristic from any mammal found in nature.

101. Based on my experience in mammalian reproduction, no mammal found in nature is a time-delayed copy of either of its parents.

102. Based on my experience cloning mammals, Applicant's clone provides an alternative, time-delayed source of nuclear genomic material of its donor mammal.

103. Based on my experience cloning mammals, this feature of Applicant's clone does not depend on the continued existence of the donor mammal.

104. Consequently, Applicant's clone can provide an alternative source of nuclear genomic material of its donor mammal, even if the donor mammal is dead.

105. The time delay of Applicant's clone allows the preservation of the genomic material of a particular mammal beyond the normal lifespan of that mammal.

106. Normally, when a mammal dies, its particular genomic composition is lost. Its progeny only contain one-half of each of its two parents' genomic material. The genomic material of one parent is inextricably scrambled together with the other parent's genomic material to create the progeny.

107. Based on my experience cloning mammals, Applicant's clone avoids this permanent loss of a particular genomic composition.

108. Thus, Applicant's clone provides the potential for the continuation of a particular genomic composition in a way that never occurs in nature.

109. Based on my experience cloning mammals, this cannot be considered a trivial difference as compared to a clone's donor mammal, which does not have this potential.

110. Moreover, Applicant's clone requires two animals, namely, a pre-existing, non-embryonic, donor mammal and a clone of that donor mammal. Based on my experience in mammalian reproduction, nature never makes such a pair of mammals.

DIFFERENCES BETWEEN SCNT CLONES AND EMBRYONIC CLONES

111. I have been asked to describe differences between a live-born clone of a pre-existing, non-embryonic, donor mammal and a clone made by the embryonic cloning procedures of a number of articles, which I will specify below.

112. By "embryonic cloning procedures," I am referring to a process of cloning using nuclear transfer starting with an embryo as the nuclear donor. In this procedure, the embryo is destroyed in the process at a time when its genetic potential is unknown.

113. I have read an article by M. Sims and N.L. First entitled "Production of Calves by Transfer of Nuclei from Cultured Inner Cell Mass Cells" in *Proc. Nat. Acad. Sci. USA* 91:6143-6147 (1994)("Sims et al. (1994)"), a copy of which is attached hereto.

114. Sims et al. (1994) reports the production of calves by nuclear transfer using cultured inner cell mass cells. (At 6143, Abstract.)

115. In Sims et al. (1994), the inner cell mass cells were generated by performing immunosurgery on *in vitro* cultured embryos. (At 6143-6144, bridging paragraph.)

116. In Sims et al. (1994), the *in vitro* cultured embryos were generated by *in vitro* fertilizing oocytes with sperm. (*Id.*)

117. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of Sims et al. (1994).

118. The calves of Sims et al. (1994) were derived using cells derived from embryos as the nuclear donor cells. (At 6143, Abstract.)

119. These embryos were destroyed during the procedure used to make the donor cells for nuclear transfer.

120. I have read an article by K.J. McLaughlin L. Davies, and R.F. Seamark entitled "In vitro Embryo Culture in the Production of Identical Merino Lambs by Nuclear Transfer" in *Repod. Fertil. Dev.* 2, 619-622 (1990)("McLaughlin et al. (1990)"), a copy of which is attached hereto.

121. McLaughlin et al. (1990) reports the production of lambs by nuclear transfer using cells from embryos at the 8- to 16-cell stage. (At 619, Abstract.)

122. In McLaughlin et al. (1990), the embryos were generated by artificial insemination with sperm. (At 619, last paragraph.)

123. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of McLaughlin et al. (1990).

124. The lambs of McLaughlin et al. (1990) were all derived using cells derived from embryos as the nuclear donor.

125. These embryos were destroyed during the procedure used to make the cells for nuclear transfer.

126. I have read an article by R.S. Prather, M.M. Sims, and N.L. First entitled "Nuclear Transplantation in Early Pig Embryos" in *Biology of Reproduction* 41, 414-418 (1989)("Prather et al. (1989)"), a copy of which is attached hereto.

127. Prather et al. (1989) reports the production of a pig by nuclear transfer using a cell from an embryo at the 4-cell stage. (At 414, Abstract.)

128. In Prather et al. (1989), the embryo was generated by breeding pigs. (At 414-415, bridging a.)

129. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of Prather et al. (1989).

130. The pig of Prather et al. (1989) was derived using a cell derived from an embryo as the nuclear donor.

131. The embryo was destroyed during the procedure used to make the cells for nuclear transfer.

132. I have read an article by Z. Yong, W. Jianchen, Q. Jufen, and H. Zhiming entitled "Nuclear Transplantation in Goats" in *Theriogenology* 35, 299 (1991)("Yong et al. (1991)"), a copy of which is attached hereto.

133. Yong et al. (1991) reports the production of goats by nuclear transfer using cells from embryos at the 4- to 32-cell cell stage. (At 299, Table.)

134. In Yong et al. (1991), the embryos were generated from pregnant goats. (*Id.*)

135. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of Yong et al. (1991).

136. The goats of Yong et al. (1991) were all derived using cells derived from embryos as the nuclear donor.

137. These embryos were destroyed during the procedure used to make the cells for nuclear transfer.

138. I have read an article by H.-T. Cheong, Y. Takahashi, and H. Kanagawa entitled "Birth of Mice after Transplantation of early Cell-Cycle-Stage Embryonic Nuclei into Enucleated Oocytes" in *Biology of Reproduction* 48, 958-963 (1993)("Cheong et al. (1993)"), a copy of which is attached hereto.

139. Cheong et al. (1993) reports the production of mice by nuclear transfer using cells from embryos at the 2- to 8-cell cell stage. (At 619, Abstract.)

140. In Cheong et al. (1993), the embryos were generated from mated females. (At 958, last paragraph.)

141. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of Cheong et al. (1993).

142. The mice of Cheong et al. (1993) were all derived using cells derived from embryos as the nuclear donor.

143. These embryos were destroyed during the procedure used to make the cells for nuclear transfer.

144. I have read an article by X. Yang, S Jiang, A. Kovacs, and R. Foote entitled "Nuclear Totipotency of Cultured Rabbit Morulae to Support Full-Term development Following Nuclear Transfer" in *Biology of Reproduction* 47, 636-643 (1992)("Yang et al. (1992)"), a copy of which is attached hereto.

145. Yang et al. (1992) reports the production of rabbits by nuclear transfer using cells from embryos at the 32- to 64-cell cell stage. (At 636, Abstract.)

146. In Yang et al. (1992), the embryos were generated by artificial insemination with sperm. (At 636, col. 2, first paragraph.)

147. Thus, sexual reproduction was used to generate the embryos used to generate the cells for the nuclear transfer procedures of Yang et al. (1992).

148. The rabbits of Yang et al. (1992) were all derived using cells derived from embryos as the nuclear donor.

149. These embryos were destroyed during the procedure used to make the cells for nuclear transfer.

150. Thus, Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) each describes clones made by embryonic cloning procedures.

151. The embryos used as the nuclear donors in the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) were generated by normal sexual reproduction.

152. Thus, the embryos used as the nuclear donors in the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) were not identical to either of its parents.

153. The embryos used as the nuclear donors in the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) were destroyed during the embryonic cloning procedures.

154. Thus, the embryos used as the nuclear donors in the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) were never "non-embryonic."

155. The non-embryonic parental mammals in Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) would have been the two parents of each of the embryos used as the nuclear donors in the embryonic cloning procedures.

156. The clones made by Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) were not clones of these mammals, since sexual reproduction was used to generate the embryos used in the embryonic cloning procedures.

157. Based on my experience in cloning mammals, the genetic complement of the clones generated by the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) would have been a mixture of the genetic complement of it two parents, and would not have had the same genetic complement as either of the parents.

158. Consequently, the clones generated by the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) could not be a live-born clone of a pre-existing, ***non-embryonic***, donor mammal.

159. Rather, the clones generated by the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991),

Cheong et al. (1993), and Yang et al. (1992) were live-born clones of a pre-existing, donor embryo, which itself was the product of sexual reproduction.

160. A live-born clone of a pre-existing, non-embryonic, donor mammal is a time-delayed, inexact copy of a non-embryonic mammal. Based on my experience in mammalian cloning, Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) did not generate such an animal.

161. A live-born clone of a pre-existing, non-embryonic, donor mammal requires two animals, namely, a pre-existing, non-embryonic, donor mammal and a clone of that donor mammal. Based on my experience in mammalian cloning, Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) did not generate such a pair of mammals.

162. In none of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) did a pre-existing, non-embryonic, donor mammal and a clone of that donor mammal exist.

163. Moreover, the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) preclude the coexistence of the clone and the donor.

164. This is due to fact that, in the embryonic cloning procedures of Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992), the embryonic donor was destroyed during the generation of the clone.

165. In contrast, a live-born clone of a pre-existing, ***non-embryonic***, donor mammal could coexist with its non-embryonic donor since the generation of such a clone does not require destruction of the donor mammal in generating the clone.

166. In my opinion, a live-born clone of a pre-existing, non-embryonic, donor mammal is not disclosed in Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), or Yang et al. (1992).

167. Prior to the birth of Dolly, as reported in Wilmut et al., Viable offspring derived from fetal and adult mammalian cells, *Nature* 385:810-13 (1997), I would have expected that the generation of a live-born clone of a pre-existing, non-embryonic, donor mammal was not possible.

168. Although Sims et al. (1994), McLaughlin et al. (1990), Prather et al. (1989), Yong et al. (1991), Cheong et al. (1993), and Yang et al. (1992) demonstrated that cloned mammals could be made using embryonic nuclear donor cells, I did not expect that it was possible, prior to the birth of Dolly, to generate a cloned mammal using non-embryonic nuclear donor cells.

169. The undersigned further declares that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: Mar. 28. 2008, By: _____
Irina A. Polejaeva, Ph.D.